Regulation of macrophage cell death proteins Mcl-1 and Bax during infection by *Mycobacterium tuberculosis*

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Background

Mycobacterium tuberculosis (*M.tb*) is an intracellular pathogen that is responsible for the infectious disease tuberculosis (TB), which is a major global health threat. In the year 2014, there were 1.5 million deaths resulting from TB. *M.tb* infects alveolar macrophages, in which it manipulates the host immune response for survival. One of the ways *M.tb* does this is by repressing apoptotic cell death. Peroxisome proliferator–activated receptor gamma (PPARγ), a master transcriptional regulator present in macrophages, is regulated by *M.tb* to ensure its growth. *M.tb* is able to avoid macrophage activation by inducing PPARγ expression, as PPARγ is a negative regulator of macrophage activation. In order to determine genes regulated by PPARγ that play a role during *M.tb* infection, the Schlesinger lab performed NanoString analysis to assess gene expression in control versus PPARγ knockdown human monocyte–derived macrophages (MDMs) infected with *M.tb*. .

PPARγ knockdown affects the expression of two regulatory proteins of macrophage apoptosis: Mcl-1 and Bax. Mcl-1 is anti–apoptotic, while Bax is pro–apoptotic. Previous studies have shown that Bax production is repressed and Mcl-1 production is induced during *M.tb* infection.

We hypothesize that during infection, *M.tb* represses apoptosis via PPARγ regulation of Mcl-1 and Bax.

Methods

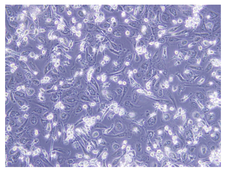

Fig 1. Microscope image of human monocyte–derived macrophages (MDMs).

- **Human monocyte–derived macrophage (MDM) preparation**

Peripheral blood mononuclear cells (PBMCs) were isolated from the blood of healthy human donors with Ficoll-Paque density centrifugation, and cultured in Teflon wells for 5 days with 20% autologous serum. Lymphocytes were removed by washing after the differentiated MDMs adhered.

- **Knockdown of PPARγ**

MDMs were transfected with 50 nM scrambled control siRNA or PPARγ siRNA using Mirus X2. After 24 hours, >90% PPARγ knockdown had occurred. MDMs were then infected or treated as described.

NanoString validation

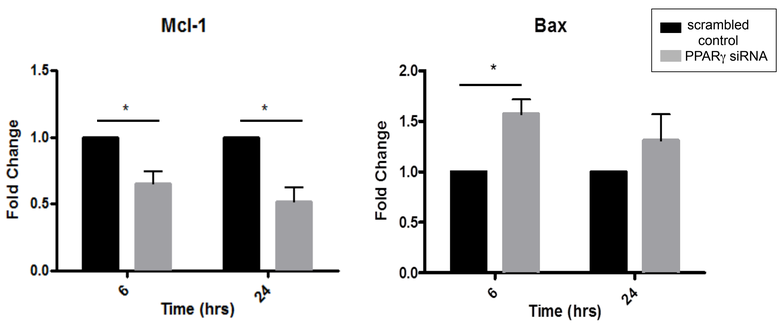

Fig 2. Fold change in Mcl-1 and Bax expression following PPARγ knockdown in *M.tb*–infected MDMs. MDMs were transfected with either scrambled control or PPARγ–specific siRNA then infected with *M.tb* at MOI 5. After 6 or 24 h, MDMs were lysed and RNA collected. The above data are qRT-PCR analysis results. Results are the mean ± SEM of 3-4 experiments. A one-way ANOVA was performed. * $P < 0.05$

Treatment with PPARγ agonists

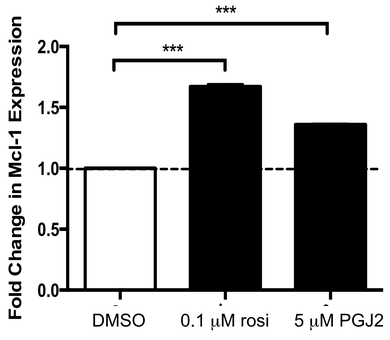

Fig 3. Mcl-1 expression increases in the presence of PPARγ agonists. MDMs were treated with two different PPARγ agonists, rosiglitazone (rosi) and PGJ2, for 24 h. Cells were then lysed, RNA collected, and qRT-PCR analysis was performed. The data from these experiments were normalized to the control group and are shown as mean ± SEM of two experiments. A one-way ANOVA was performed. *** $P < 0.001$

M.tb infection

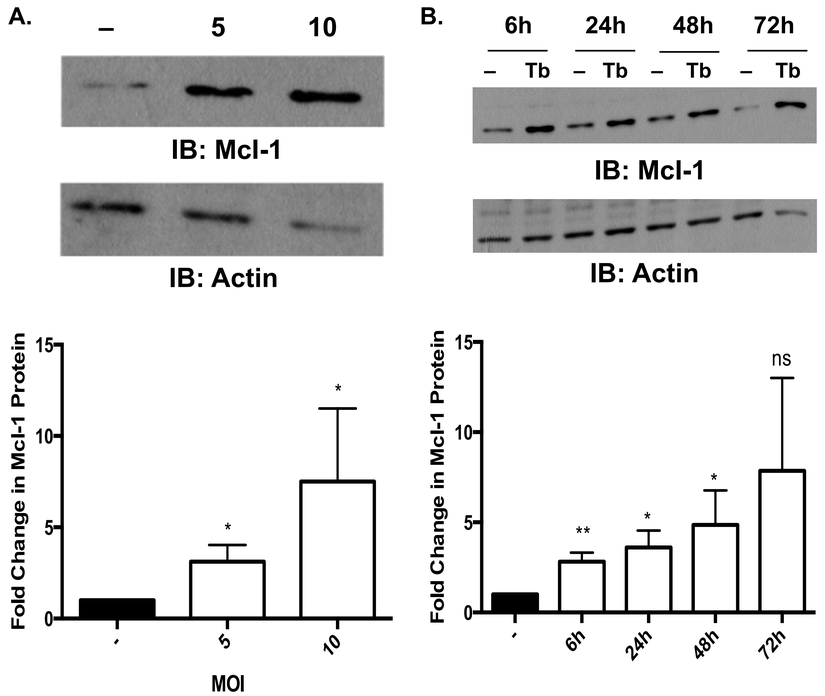

Fig 4. Effects of Multiplicities of Infection (MOIs) and time on Mcl-1 protein amounts during *M.tb* infection. Cells were either uninfected, or infected with *M.tb* (Tb) for 24 hours at MOI 5 or 10 (**A**) or 6, 24, 48, or 72 hours at MOI 5 (**B**). Cells were then lysed and Western blot was carried out. Densitometry analysis was performed with Image J. These results are the mean ± SEM of 3-6 experiments. A one-way ANOVA was performed. * $P < 0.05$, ** $P < 0.01$

PPARγ knockdown

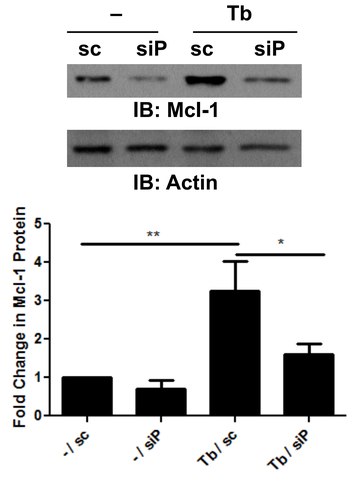

Fig 5. Western blot analysis of Mcl-1 with *M.tb* infection and PPARγ knockdown. MDMs were either transfected with scrambled control (sc) or PPARγ (siP) siRNA, then subjected to *M.tb* infection for 24 hours. The cells were then lysed, and Western blot was carried out. These results are the mean ± SEM of 5 experiments. A one-way ANOVA was performed. * $P < 0.05$, ** $P < 0.01$

Model

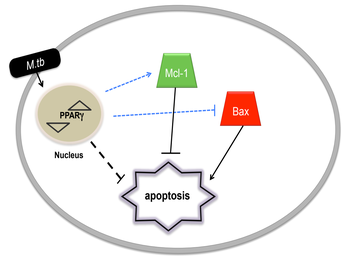

Fig 6. Model. It is known that *M.tb* induces PPARγ during infection, and that Mcl-1 and Bax are both apoptosis regulators that are controlled by *M.tb*. However, how *M.tb* regulates Mcl-1 and Bax is unclear. Our preliminary data show that Mcl-1 and Bax are genes regulated by PPARγ during *M.tb* infection. We hypothesize that, by controlling PPARγ, *M.tb* is able to in turn regulate the expression of Mcl-1 and Bax to repress apoptosis.

Conclusion

- We have identified Mcl-1 and Bax as novel potential effectors of PPARγ during *M.tb* infection.
- If PPARγ is stimulated with a chemical agonist, Mcl-1 expression increases. PPARγ knockdown results in a decrease in Mcl-1 expression.
- Similarly, if PPARγ is stimulated with *M.tb*, there is a significant increase in Mcl-1 protein in a MOI– and time-dependent manner. PPARγ knockdown results in a decrease in Mcl-1.
- Our findings contribute new knowledge of how *M.tb* alters the human innate immune response to continue its survival within the macrophage.